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SIGNIFICANT ACCURACY IN RECORDING GENETIC DATA

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In 1913, I contributed a paper to the Botanical Gazette (**55**: 177–188) on the inheritance of flower size in a cross between *Nicotiana alata grandiflora* Comes, and a type thought to be *Nicotiana forgetiana* Hort. Sand. Corolla size had been selected for study because in this genus it is "so comparatively constant under all conditions attending development"—something which could not be said of any other size character that had been under observation. Since other investigations of the same kind were under way, and a larger amount of data might be reported later, the "liberty of asserting the truth of this statement" with only the following data in its support was requested. This paragraph followed.

"During the past four years, I have grown about 20 species of Nicotiana in considerable numbers. They have been grown under very diverse conditions. Some have been starved in four-inch pots, others have had the best of greenhouse treatment; some have had poor field conditions, others have had all field conditions practically at their best. The height of the plants, the size of the leaves, and similar size complexes have varied enormously, but the size of the corollas has scarcely varied at all. For example, plants of *Nicotiana sylvestris* Speg. & Comes, grown to maturity in four-inch pots, produced no leaves longer than 7 inches. On the other hand, sister plants of the same pure line produced leaves 30 inches long in the field. Both series, however, produced flowers with the same length and spread of corolla. Furthermore, cuttings from 20 of the field plants reported in this study were rooted and grown in small pots (6 inch) in the greenhouse. Their blossoms were the same size as those of the field grown plants from which they came."

[The **Journal** for April (**3**: 135–210) was issued April 18, 1916.]

Recently Goodspeed and Clausen have published in this Journal (2: 332–374. 1915) an immense amount of data on the influence that certain environmental factors have on flower size in Nicotiana. The conclusions they draw are eight in number based upon 25,000 measurements of the length and spread of the corollas of *Nicotiana tabacum* var. *macrophylla* and three hybrids between *N. tabacum* varieties and *N. sylvestris*, and run somewhat as follows:

1. Both length and spread of corolla decrease during the flowering season to such an extent that at the end of six weeks the average spread may drop 6 mm. and the average length 4.5 mm.

2. The F_1 *N. tabacum* × *N. sylvestris* hybrids are short-lived perennials, and the flowers of the second season are of approximately the same size as those of the first season.

3. Removal of open flowers during the normal flowering season prevents nearly all decrease in size.

4. Flowers apparently fully opened are smaller before than they are after anthesis, even though the anthers are partially sterile.

5. Flowers on pot-grown cuttings are smaller than those borne on the field plants from which they were taken.

6. Under favorable and unfavorable greenhouse conditions, flower size varies distinctly and in the same direction as the vegetative characters.

7. Length of corolla is more stable than spread of corolla under environmental stimuli.

8. "The only true distribution representing the flower size of a population must be based upon measurements which, for each plant, extend over the greater part of the period of flowering normal for the given species or hybrid group, or cover an identical portion of the flowering period of each plant."

The data were collected and these conclusions drawn, the authors say, "to establish tentative criteria in keeping with which flower size investigations, in Nicotiana at least, should be carried on and interpreted."

The statements of Goodspeed and Clausen and those quoted from my own paper seem at first sight to be irreconcilable. Indeed, the authors have done me the honor of devoting a considerable portion of their paper to criticizing my views and methods. For example, because it was maintained that flowers are constant under different environments compared with the changes exhibited by vegetative

organs, they have assumed that no precautions whatever were taken to eliminate environmental differences. Since the statement was made that plants were grown under diverse conditions, a fact mentioned merely in connection with the question of the effect of stimuli on corolla size, they seem to have concluded unjustly and unreasonably that the data from *these* experiments were used in the paper under consideration.

On the other hand, Goodspeed and Clausen are perfectly justified in asking for a description of the way in which my data were taken. I wish to make such a statement, therefore, in order to support my former paper and some other studies on the inheritance of flower size which are to be published in the near future, and because of the opportunity presented to illustrate a question of considerable general interest. This question, which as a teacher of genetics I have found neglected by research students more than any other, is: *What is significant accuracy in recording data?*

The seemingly opposed statements of Goodspeed and Clausen and of myself serve to illustrate the thought in mind. The two allegations are not wholly discordant. Although I do not wish to withdraw or to modify my own statements, at the same time I am willing, in a broad sense, to accept most of their conclusions. Excluding certain differences in our data that are undoubtedly due to dissimilar conditions at Berkeley, California, and at Boston, Massachusetts, my own results are similar to theirs except as to the magnitude of the changes caused by environmental differences. The point upon which we differ decidedly is the *significance* of the results in relation to the problem at hand—the inheritance of differences in corolla size in Nicotiana.

One of my college instructors once said to me: "It is seldom necessary, in the interests of scientific accuracy, to weigh a ton of hay on an analytical balance." That statement might be made the basis of a course on Precision of Measurements. One is hardly ever required to impress mechanical accuracy upon really earnest students. They will weigh and measure material with the utmost pains (in spirit at least). What is difficult is to impress an idea of true precision. It is not uncommon to see measurements recorded to tenth millimeters after the random use of two scales having a one percent difference, or material for analysis weighed to the fourth decimal place with weights that have never visited the Bureau of Standards, on a

balance with very unequal arms. It is rare to find students who think of these errors and endeavor to correct them, although such correction is as necessary in biology as in physics. Let us see how our biological problem fits the rules for the treatment of errors in use in experimental physics.

It was desired to record, in such a manner that they would be comparable, numerics that represented the phenotypes of series of plants of species of Nicotiana in regard to corolla length and spread, sufficiently accurately that genetic analysis of the results might be made.

The investigation was initiated by a series of preliminary measurements designed to show the practical physical limits to the precision of the direct measurements. Repeated measurements of the same flowers showed that there were residual errors beyond one millimeter in the case of length and two millimeters in the case of spread of corolla. Measurement to millimeters was adopted, therefore, although these measurements were afterwards thrown into larger classes for reasons that can be justified biometrically.

Then came a study of ontogenetic variation in order that the factors affecting such variation might be detected. The factors that would naturally occur to anyone who had had experience in growing plants were time of planting, physical and chemical condition of the soil, moisture, age of plant, flowering period, age of flower, position of inflorescence on plant and position of flower in the inflorescence. To determine the effect of each of these factors, it was necessary of course to eliminate the influence of all the others as far as possible. Since the cultures to be compared were nearly always planted at the same time, and since this variable is somewhat dependent upon others that were under consideration, it was neglected. My cultures have also been grown in well-drained soil very uniform in its fertility, but it was thought wise to determine how much effect extreme soil conditions might have. Several species growing outside in soil of good tilth were compared with greenhouse pot cultures. Three-inch, four-inch, five-inch and six-inch pots were used in various species, but the treatment was uniform for each species. The species were *N. tabacum* (several varieties), *N. rustica* (several varieties), *N. longiflora* (two varieties), *N. sylvestris*, *N. paniculata*, *N. acuminata*, *N. forgetiana* and *N. alata grandiflora*. Since only from ten to twenty plants could be grown in the greenhouse in most cases, statistical constants were not calcu-

lated, for I have not the faith of Goodspeed and Clausen in probable errors based on nine or ten observations (see their tables II *a*, *b* and III *a*, *b*). Averages of five flowers per plant taken when first in full flower, however, indicated means within a millimeter of each other for length and within two millimeters of each other for spread of corolla for over half of the species, when compared with the sister plants in the field. The greatest difference was in a *N. alata grandiflora* test where the starved plants showed an average of about 5 mm. shorter and 7 mm. narrower flowers. Hybrids were also tested. As I do not consider it necessary to cite figures endlessly where they serve so little purpose, however, only a table of results on a cross between two varieties of *N. longiflora* is given, the field records and the pot records being made by different observers. The general

TABLE I

Frequency Distribution for Length of Corolla in Cross between N. longiflora Varieties

Designation	Class Centers in Millimeters																				
	37	40	43	46	49	52	55	58	61	64	67	70	73	76	79	82	85	83	91	94	97
No. 383 field..	4	32	1	...	...	...	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
No. 383 pots..	3	15	1	...	...	...	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
No. 330 field..	.	...	.	...	...	...	...	...	...	...	...	...	...	...	...	...	.	5	7	10	2
No. 330 pot...	.	...	.	...	...	...	...	...	...	...	...	...	...	...	...	...	.	1	4	3	1
(383 × 330) F_3A field....	.	...	.	...	...	...	...	...	...	4	20	25	59	41	19	2	.	.	.	...	.
Ditto, pot.....	.	...	.	...	...	...	...	...	...	...	1	3	4	1	...	...	.	.	.	...	.
(383 × 330) F_3B field....	.	...	6	20	53	49	15	4	...	...	...	...	...	...	...	...	.	.	.	...	.
Ditto, pot.....	.	...	.	2	6	4	...	...	...	...	...	...	...	...	...	...	.	.	.	...	.
(383 × 330) F_3C field....	.	...	2	3	9	25	37	70	19	10	...	...	...	...	...	...	.	.	.	...	.
Ditto, pot.....	.	...	.	...	...	2	3	3	...	...	...	...	...	...	...	...	.	.	.	...	.
(383 × 330) F_3D field ...	.	...	.	...	...	...	...	...	2	8	14	21	39	39	32	10	1	.	.	...	.
Ditto, pot.....	.	...	.	...	...	...	...	...	...	...	1	...	3	3	2	3	.	.	.	...	.
(383 × 330) F_3E field....	.	...	.	...	1	...	1	1	1	2	16	33	43	34	20	6	1	.	.	...	.
Ditto, pot.....	.	...	.	...	...	1	1	...	...	...	...	1	4	1	2	...	.	.	.	...	.

effect of starvation can be seen even without having the means calculated. A comparatively small number of observations were made on each population, but they serve as samples of the frequencies found. Certainly no marked decrease in size is apparent, and since the vegetative organs of the pot-grown plants varied from one half to one fifth the size of those in the field (linear dimensions), it seems that one

should be justified in stating that comparatively starvation had no effect on the flowers.

Both sets of these plants had a sufficient supply of moisture to keep them healthy. When this is not the case there is some difference in flower size. For example, some *N. rustica* plants each showing a mean flower length of 20 mm. with extremes of 18 mm. and 22 mm. at the first of the season, decreased in their mean flower length to 18.8 mm. after being in flower for four weeks during which very little rain fell. Then came four inches of rain within forty-eight hours. After this, stout vigorous laterals arose from the lower part of the main stems bearing flowers with a mean length of 21.1 mm. (extremes were 19 mm. and 23 mm.). Thus a marked difference in activity of cell division shows its effect on the flower.

This factor is probably the cause of the greater size shown by flowers on lateral branches when compared with those on terminal branches in Goodspeed's and Clausen's work (Tables XIII, XIV, XV). These authors also found that the flowers on new vigorous branches after "cutting back" were increased in the same way.

These facts should be taken into consideration when examining the conclusion of the California botanists that flower size decreases markedly as the length of the flowering season increases. Their data, as well as my own, proving that flower size may keep up to that of the first of the season and even increase if the weather conditions remain favorable for the production of vigorous new lateral branches, show that it is questionable whether a significant decrease in flower size occurs during the time that data would be likely to be taken. Their data showing marked decreases from the first of the season to mid-season are from populations of 9 and 10. During similar periods I have found no measurable decrease in flower length in *N. tabacum*, *N. longiflora*, *N. paniculata* and *N. rustica*. I have found a mean decrease of 1.0 mm. to 1.5 mm. which possibly is due to this factor in certain cultures of *N. langsdorffii*, *N. acuminata*, *N. forgetiana* and *N. alata grandiflora*, but I think the true occasion of the decrease was lack of moisture. On the other hand, there seems to be evidence in Goodspeed's and Clausen's data that toward the end of the season there is likely to be a decrease in flower size. My own data have shown a drop of from 4 mm. to 8 mm. in both corolla length and spread in various species in the last dozen or two flowers produced. This shows as a sudden change which is evidently due to physiological

reasons. The true state of affairs is masked, therefore, when this decrease is treated as a gradual drop in flower size during the season. If measurements on greenhouse cultures grown in proper sized pots are taken daily over a long period, they simply show comparative uniformity in flower size until about the end of the flowering season. Then a decrease which produces a sharp bend in the curve occurs.

As to variation in size owing to age of the flower, I have found that this is largely a mechanical difficulty. There is no difference in length between flowers before and after anthesis, for anthesis takes place normally either before or within 10 hours after the flower opens in all species of Nicotiana under Boston conditions. A flower if unpollinated may open for as many as 5 successive days, and there is a slight increase in both length and spread of the corolla. But a pollinated flower seldom opens on more than two successive days. The flower becomes less firm however and the *spread* of the corolla may *appear* to increase.

Flowers of the same relative position on vigorous branches are the same size whether they be on the main stalk or on laterals in species like *N. forgetiana* and *N. alata grandiflora* which are characterized by vigorous lateral branches from the base of the stem. Flowers on lateral branches in species like *N. tabacum* where the main stem is so much more vigorous, average (in my counts) slightly less (under 1 mm.) than those on the main stem.

After about the sixth flower on the species having racemes, and on the flowers coming out after the first full glory of the panicled species, there is also a slight decrease in size owing to decrease in the conducting channels of the fibro-vascular system.

What information do these observations, which are the preliminary "qualitative" tests made in every investigation, give us? They show that to record the phenotypes of flower size of a series of Nicotiana plants, the seeds should be sown at the same time in uniform soil, the plants should be pricked out uniformly and set at the same time in a plot of uniform fertility. The flower records should be made within two or three weeks of each other at the first of the season, allowing no marked climatic change to intervene if possible. The flowers recorded should be the vigorous flowers (as stated in the last paragraph) of vigorous branches, and should be measured on the same day that they open.

This procedure should be followed where it is physically possible,

and any departure noted in order that a correction for any constant error due to it may be calculated, if it be advisable. But, one might ask, would not any trained geneticist have taken these precautions anyway? What has been gained?

The advantages are real. Unsuspected constant errors often come to light through such preliminary investigations. The good fortune that none appeared here certainly makes it no less satisfactory. It showed that control of conditions in such a manner that constant errors will be negligible in the end result is technically possible. It gave a definite idea of the magnitude of the error produced when various environmental factors do vary, and this is very necessary in determining the probable limits of error.

There is a way of testing the conclusion that with the conditions controlled as suggested the constant error is negligible. If the same plants are measured during *similar portions* of successive periods of flowering activity, there is but one other obvious variable—total age of plant. If the latter has no measurable effect the two frequency distributions should duplicate. On this point I have no data, but Goodspeed and Clausen have corroborated the expectation in their conclusion number two. I do have some data on random samples of the same pure line grown in different years. This will be taken up later, however, as another point is involved.

Now the question arises: If records are made in this uniform manner, how many records from each plant are needed to obtain a measure of that plant with the precision necessary for a genetic investigation? Goodspeed and Clausen say that twenty-five flowers is the minimum. At the beginning of my Nicotiana investigations (1908), I used the same number, curiously enough. But I soon found that this was "accuracy with no significance," and the number was reduced to five. I now use but one measurement per plant. This is done because the precision is so nearly that of using twenty-five flowers, that it would be a waste of labor to try to attain the other. Furthermore the precision obtained by measuring twenty-five flowers is only appreciably greater when it can be done in a short time, otherwise *constant* errors may become very much greater.

The precision attained by measuring one flower per plant is all that is required for the use to which the data are to be put, and it is a rule of experimental physics not to strive for greater accuracy.

This matter can and has been tested in two ways. The first is to

compare random frequency distributions of the corolla size of single plants with frequency distributions of the flowers when selected from vigorous branches and measured on the same day they have opened. This procedure gives a measure of the accuracy of single flower selections. To illustrate this, data from two species with very different sized flowers are submitted.

TABLE II

Comparison of Random Samples of Corolla Length on Single Plants and Samples in which Constant Errors have been Largely Eliminated

Name	Class Centers in Millimeters											
	20	21	22	23	24	25	26	27	28	29	30	31
N. paniculata, Random	...	...	...	...	1	3	16	2	3	...	...	....
" " Selected	...	...	...	...	...	4	18	3	...	...	...	....
" " Ran.	...	...	...	2	4	14	4	1	...	...	...	....
" " Sel.	...	...	...	...	5	17	3	...	...	...	...	....
" " Ran.	...	...	...	...	...	...	4	16	5	...	...	....
" " Sel.	...	...	...	...	...	...	3	20	2	...	...	....
" " Ran.	...	...	...	2	3	15	4	1	...	...	...	....
" " Sel.	...	...	...	...	3	22	...	...	...	...	...	....

Name	Class Centers in Millimeters											
		70	73	76	79	82	85	88	90	94	97	100
N. alata gr., Ran.	...	...	...	1	3	16	4	1	...	...	...	....
" " " Sel.	...	...	...	...	2	22	1	...	...	...	...	....
" " " Ran.	...	...	...	...	1	6	14	3	1	...	...	....
" " " Sel.	...	...	...	...	...	3	18	4	...	...	...	....
" " " Ran.	...	...	...	...	...	2	3	17	3	...	...	....
" " " Sel.	...	...	...	...	...	...	2	23	...	...	...	....

These plants are among the most uniform and the most variable respectively, and give an idea of the range of variability involved.

The other test made was to select fifteen flowers on a plant at random, and determine the mean to the nearest millimeter; then to find the deviation from this mean when single flowers were selected. In 100 tests of flowers shorter than thirty millimeters 88 selections were made within the 3 millimeter class to which the mean belonged. The remainder were in contiguous classes. On flowers between 70 and 100 millimeters long 82 out of 100 selections were within the 6 millimeter class to which the mean belonged. The remainder with 2 exceptions were in contiguous classes.

From these tests it will be seen that the probable error of the selection (equal chances) is not over plus or minus 2 percent. If this

were a constant error it would be considerable. But it must be remembered that it belongs to the class of accidental errors and that in the long run the minus errors are compensated by the plus errors.

Such compensation can be clearly seen and the accuracy of the method perhaps most clearly demonstrated by comparing frequency distributions of the same pure line, daughters of the same plant, during successive seasons. In a number of cases populations of sister plants were grown for two and three years. The seed in each case came from single 1909 or 1910 plants, and since the percentage germination remained practically constant, the different populations are in the nature of duplicate and triplicate determinations. If then the frequency distributions are sufficiently alike that they may be presumed to be random samples of one population, the method is accurate enough for genetic purposes. A sample of the result is shown in Table III.

TABLE III

Random Samples of the Same Population Grown in Different Seasons

Name	Class Centers in Millimeters												Means
	34	37	40	43			85	88	91	94	97	100	
N. longiflora, var. *A*, 1911	...	13	80	32	...	...	...	...	...	...	...	...	40.46±.11
" " " " 1912	1	4	28	16	...	...	...	...	...	...	...	...	40.61±.19
" " " " 1913	...	4	32	1	...	...	...	...	...	...	...	...	39.76±.12
N. longiflora, var. *B*, 1911	...	...	...	...	...	...	...	6	22	49	11	...	93.22±.16
" " " " 1912	...	...	...	...	...	...	...	2	16	32	6	1	93.37±.20
" " " " 1913	...	...	...	...	...	...	...	5	7	10	2	...	92.12±.37

When one takes into consideration the difference in size of corolla among Nicotiana species and varieties that will cross and give fertile hybrids—*i. e. N. langsdorffii* 21 mm. and *N. alata grandiflora* 85 mm., it is scarcely necessary to enter into a biometrical argument on the precision of the method. Here are two small samples of the same population of *N. langsdorffii* grown in 1911 and 1914:

Designation	Class Centers in Millimeters				
	19	20	21	22	23
1911 plants	...	3	12	1	2
1914 plants	1	9	33	7	1

Can it be doubted that the phenotype for corolla length to which *N. langsdorffii* belongs is shown here with an accuracy much greater

than is necessary when an analysis of the hybrid progeny of it and *N. alata grandiflora* is contemplated? Biometrical methods are much too imperfect to demand more. There is no intention to discuss here the reasons why the biometrical methods in general use in genetics are imperfect. But it must be emphasized that they are merely used in default of better, since many of them cannot be defended either mathematically or biologically. For example, common sense tells us that equal-sized classes should not be used for the two very different species shown in Table III, where the corolla of one is three times that of the other, yet no satisfactory method has been proposed which does away with the difficulties involved. Since it is necessary to use such poor methods in calculating our end results in genetic studies of size, however, one should remember that labor to record data far more precisely than these methods require is *labor wasted*.

At the same time, though one may believe that biometrical methods are imperfect for certain purposes, they are founded on the theory of probability and when used should be used with this in mind. Having recorded his data with the precision desired, one should not try to analyze them until he has collected a sufficient number of observations to make calculations of residual errors have meaning. Just what the minimum number should be varies with the problem and cannot be discussed in this paper. There are several textbooks on the Theory of Measurements in which the matter is treated in detail. All I wish to point out here is that in every problem capable of biometrical analysis there is such a minimum, and if the data to be analyzed are far under this required minimum, no over precision (in cases where this is possible) in making the records will give them value.

An excellent illustration of this is found in Goodspeed's third article on Quantitative Studies of Inheritance in Nicotiana Hybrids.[1] The author used his method of recording measurements of flowers through a considerable portion of the flowering season in order to determine the phenotypes to which the plants belong, and yet has made analyses of frequency distributions having such a small number of entries that they possess no meaning whatever. Among 44 frequency distributions, 29 have less than 12 plants recorded. He recognizes the fact that the number of plants involved is too small, but feels that this deficiency is balanced by the accuracy of his records.

[1] Univ. Cal. Pub. Bot. 5: 223–231. 1915.

He says: "Data which have been submitted, however, leave no room for doubt in my own mind that investigations on the inheritance of flower-size demand the recognition of certain definite criteria and that the results of such investigations are vitally influenced by inherent as well as externally induced physiological states peculiar to the plant. Thus it remains to be seen if as many as 800 plants are necessary to establish the validity of an expanded Mendelian notation in F_2 of a flower-size hybrid, whether the 40,000 to 80,000 measurements, seemingly essential to a fair expression of results, can be accumulated. In other words, the experiment with which this paper deals has been a partially successful effort to measure many flowers on a few plants with the thought that the conception of flower-size would thus be approximately perfect for a few, rather than certainly imperfect for many plants. It is undeniably true that the number of plants is smaller than it should be, and it is perfectly evident that if the flowers on a larger number of plants cannot be correctly measured the attempt is not worth making."

One could hardly find a better illustration of "accuracy without significance." These views are absolutely indefensible mathematically. It has been shown that the method used by Goodspeed in making his records has only a fallacious claim to great precision; but, granting that the method is extremely accurate, it is an accuracy unnecessary to the end result. On the other hand, it should be clear that records in sufficient number to make probable errors significant is positively essential for a biometrical analysis. This end can only be attained by recording larger numbers of plants and not by over-refinement in the plant records. The plant records should have the precision required by the end result, but greater precision does not influence this result.

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